0.75–0.965 for S/P values ranging from 0.0515 to 0.400.

All protein–salt mixtures (samples 1–7, Table I) have plots similar in shape to that of salt; they adsorbed water above an a_w of 0.75 and little moisture below that point. Figures 3 and 4 indicate that as the salt content increased, the slope of the lines increased above an a_w of 0.75 with increasing salt content (Table III). The isotherms approached that of pure salt as the salt content increased. Thus, salt content was the most important factor controlling the equilibrium moisture in this study.

The present technique of starting with dialyzed muscle proteins and adding known amounts of salt before equilibration to known a_w has enabled us to quantitate the effect of salt on water binding in both desorption and adsorption modes. Although the dialyzed protein isotherms did not show a hysteresis effect, the isotherms of the salt–protein mixtures showed strong hysteresis effects with the desorption isotherms having the higher moisture contents. The effect of hysteresis became larger with increasing salt content. This suggests that the hysteresis effect observed for the salt–protein mixtures was due to the salt, which showed a large hysteresis effect by itself.

An interesting application of these data is to assume that a processed meat contains 3% total salt and 20% protein (S/P = 0.15) and that preservation is assured at $0.85a_w$. Figure 4 shows that meat can contain as much as 1.1 g of water/g of solids with this salt content and be shelf stable. Thus, the above addition of salt to the processed meat with an additional adjustment of the moisture content enabled preservation while retaining almost 3 times as much water.

Registry No. NaCl, 7647-14-5; $ZnSO_4$, 7733-02-0; $BaCl_2$, 10361-37-2; $(NH_4)_2SO_4$, 7783-20-2; water, 7732-18-5.

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Effects of Drying on Selected Qualities of *Spirulina platensis* Protein

John D. Baranowski,* Carlos A. Dominguez, and Paul C. Magarelli

Spirulina platensis was collected after culture and dried in four ways: freeze, drum, cabinet, and solar. Available protein lysine was increased in the treatments involving drying at elevated temperatures, and in vitro enzymatic protein digestibility was increased in all samples over that of an undried control.

The production of alternate sources of protein for human and animal needs has become increasingly important. *Spirulina platensis* is a blue-green algae (Cyanobacterium) that has been found to exceed 50% crude protein (Anusuya Devi et al., 1981). A closely related species, which may actually be synonymous with *S. platensis*, is *Spirulina maxima*, which has been found to contain from 60 to 71% crude protein. This is a high-quality protein, with a digestibility of 76–84%, a protein efficiency ratio (PER) of 2.2–2.6 (74–87% that of casein), and a biological value (BV) of 60–72 (Clement et al., 1967; Skowronski, 1980). The use of *Spirulina* as a high-protein feed supplement has recently been identified by the State of Hawaii as a research priority (Anonymous, 1982).

After collection of the *Spirulina*, the raw product is approximately 90% moisture, which presents a storage problem in terms of both bulk and perishability. These two problems are easily solved by drying. However, drying can induce changes in the quality of the protein (Wolf et al., 1978; Adrian 1975). This study was undertaken to

Department of Food Science and Human Nutrition, University of Hawaii at Manoa, Honolulu, Hawaii 96822 (J.D.B. and C.A.D.), and The Oceanic Institute, Makapuu Point, Waimanalo, Hawaii 96795 (P.C.M.).

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determine the effects of a number of drying methods on the quality of *Spirulina* proteins.

MATERIALS AND METHODS

Fresh *Spirulina* was hand-harvested from 10 000-L fiberglass culture tanks at the Oceanic Institute, Waimanalo, HI, by using a 41 μm mesh net. The resultant moist product was held at 4 °C while awaiting processing.

Cabinet-Drying. Approximately 250 g (wet weight) of *Spirulina* was spread to a depth of 0.3 cm on aluminum foil. These were dried in a Precision Scientific Group (GCA Corp., Chicago, IL) mechanical convection oven at 130 °C. The air volume control was at the "1" setting.

Drum-Drying. The *Spirulina* was made into a slurry by diluting the cells ca. 1:1 (v/v) with distilled water. Drying was accomplished by using a laboratory counter-rotating double drum dryer. The drums were 19.5 cm long with 15-cm diameters. The actual drum contact time was approximately 10 s. The outlet steam pressure was 25 psig, equivalent to ca. 130 °C.

Freeze-Drying. Two hundred and fifty grams of the cells was frozen to −30 °C prior to freeze-drying. The shelf temperature was held at 25 °C throughout the drying.

Solar-Drying. *Spirulina* was spread to a depth of 0.3 cm on aluminum foil for solar-drying. The apparatus used as an SB-1 combined mode solar dryer (Moy et al., 1980). This combines the use of direct solar radiation on the product and the use of a solar collector to furnish preheated air to the dryer itself. Temperatures inside the apparatus at the drying site ranged from 47 to 58 °C over the drying period.

Moisture Determination. The moisture content of the fresh cells and residual moisture in the dried preparations were determined in triplicate by using the AOAC method (1975).

Preparation of Protein Samples. Protein extracts were prepared by using the procedures of Anusuya Devi et al. (1981) with the following modifications. Extractions of all dried samples were carried out, without hexane extraction of the sample, using a solute:solvent ratio of 1:10, 1:7.5, and 1:7.5 (dry cell weight:water, pH 8.0) for the three successive extractions. Each of these was centrifuged at 10000*g* for 10 min. Supernates from the three extractions were combined and centrifuged at 15000*g* for 10 min to remove any residual cell material. Cell disruption for all dried samples was by hand grinding.

Fresh cells were diluted with pH 8.0 water (30 g plus 30 mL) and ruptured by using a French pressure cell at 5000 psi. The two successive extractions were each done with 30 mL of pH 8.0 water. The extracts were centrifuged, the supernates were combined, and the final solution was centrifuged by using the same methods as for the dried sample extracts. Protein solutions were dialyzed (6000–8000 M_r cutoff) against 0.01 M phosphate buffer, pH 7.5, for 48 h. Protein concentrations were determined by the Lowry method using bovine serum albumin, fraction V, as a standard.

Available Lysine. Available lysine was determined by the fluorescent technique of Goodno et al. (1981), using *N*-acetyllysine (Sigma Chemical Co.) as a standard. Samples were analyzed in triplicate.

In Vitro Digestibility. A protein quality index for the extracted proteins was determined by using the method of Akeson and Stahmann (1964). Ten milligrams of protein in 4.0 mL of 0.01 M phosphate, pH 7.5, was incubated with 0.60 mg of pepsin (Sigma, P-7000, containing 25% protein) in 1.5 mL of 0.1 N HCl at 37 °C for 3 h. After being neutralized by adding 0.75 mL of 0.2 N NaOH, further digestion was accomplished by the addition of 0.4 mg of pancreatin (Sigma, P-1625), in 0.75 mL of 0.1 M phosphate buffer, pH 8.0. The samples were then incubated at 37 °C for an additional 21 h. Enzyme action was halted by immersing the mixtures in an ice bath. Undigested protein was precipitated by the addition of an equal volume of cold 10% trichloroacetic acid (TCA). The samples were mixed and centrifuged at 10000*g* for 10 min. The precipitates were dissolved in 0.2 N NaOH and brought to volume. Protein concentrations were then determined by the Lowry method. A series of controls, containing only the digestive enzymes, were treated in the same manner. Total precipitated protein was determined by mixing all reagents and protein solutions at one time followed by immediate precipitation with TCA. These were then treated in the same manner as the digested samples with respect to protein determination. Each sample was analyzed in duplicate.

Table I. Moisture in Fresh and Dried *Spirulina*

	% moisture[a]
fresh	89.9
forced-air cabinet at 130 °C	
30 min	78.0
60 min	20.2
90 min	2.0
drum-dried	4.4
freeze-dried	0.6
solar-dried	
2 h	82.0
9 h	6.3

[a] Wet weight basis.

Table II. Effects of Drying Method on the Lysine Availability and Digestibility of Extracted *Spirulina* Protein

	lysine[a]	digestibility
fresh	30.0	65.9
freeze-dried	27.5	85.2
solar-dried	38.5	85.6
drum-dried	41.9	89.2
cabinet-dried	41.5	72.0

[a] Mol of lysine/100 000 g of protein.

RESULTS AND DISCUSSION

Spirulina is a protein source that is very high in moisture but is readily dried to a compact product (Table I). . Although there are concerns about the deleterious effects of heat treatments on protein quality, drying of the cells did not have adverse effects on available lysine or in vitro protein digestibility (Table II). Available lysine ranged from 4.02 to 6.06% of the extracted proteins. This is lower than the 7.06% lysine for whole cells as measured by amino acid analysis (Baron, 1975) but is higher than an amino acid analysis performed more recently (1.77%; Ako, 1983). These values are also higher than those measured by Adrian (1975), who found 2.97, 2.15, and 1.80% lysine for freeze-dried, spray-dried, and drum-dried cells, respectively, using a *Leuconostoc mesenteroides* bioassay.

It is apparent from the data in Table II that the heat-treated samples actually show an increase in available lysine. These results are contradictory to those of Adrian (1975), who found a clear decrease in lysine in dried cells, although whole cells and not extracted proteins were analyzed. The increase in available lysine in the heat-treated cells in this study may be due to different protein profiles for these samples than for the fresh and freeze-dried cells. *Scenedesmus* cells that were drum-dried were noted to have a higher digestibility than fresh cells (Subbulakshmi et al. 1976), which was explained as a disruption of the cell wall, making protein more available. While *Spirulina* does

not have a cellulosic cell wall, it does have a cell membrane that may be tying up protein of higher available lysine, which is being liberated with drying. Although some of these are rather severe heat treatments, the absence of any appreciable quantity of reducing carbohydrates "protects" the lysine from the expected Maillard reaction losses (Adrian, 1975).

While the available protein lysine was increased in the heat-treated samples, no clear correlations were noted between in vitro protein digestibility and heating. Protein in all dried samples was more digestible than proteins extracted from wet algae. The results for the proteins of freeze-dried, solar-dried, and drum-dried cells are comparable to those of Bouges (1972) and Durand-Chastel (1972) as reported by Lipinsky and Litchfield (1974). These reports provide evidence for 84% digestibility for dried *Spirulina maxima* cells but contradict those reported by Anusuya-Devi et al. (1981). They found that proteins from freeze-dried and sun-dried *Spirulina platensis* had a digestibility of ca. 70%, while fresh cell proteins were roughly 83% digestible. While essentially the same in vitro assay was used here as in their study (24-h incubation vs. 27 h, respectively), they started with *n*-hexane-extracted cells. Also, they measured nondigested proteins by micro-Kjeldahl assay of TCA-precipitated proteins, while in this study the precipitable proteins were dissolved in base and analyzed by using the Lowry method.

Even with the differences noted for results from other studies, the work herein shows that *Spirulina* cells may be dried in any number of ways, with little, if any, loss in protein digestibility or available lysine.

Cabinet-dried *Spirulina* was used as a dietary protein source in two separate nutrition trials. For both poultry and marine shrimp, growth and survival were equivalent to control animals that were fed diets based on more traditional sources of protein (Magarelli, 1984).

Registry No. Lysine, 56-87-1.

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Protein Polymerization in Red Cell Membranes

Jorge O. Bouzas and Marcus Karel*

Human red cell membranes were used as a model of an organized lipid protein system to investigate protein damage during storage under dry conditions and controlled temperature, under air and nitrogen, and after various physical and chemical treatments. It was found that these treatments affected protein polymerization at time 0 and during storage. Enzyme damage depended not only on treatment and storage condition but also on location within the membrane. Acetylcholinesterase, an externally oriented enzyme, lost all its activity when treated with chloroform while retaining most of it when membranes were only freeze-dried or sonicated and freeze-dried. During storage no further changes were observed. G3PD, an enzyme bound to the inner membrane surface and oriented toward the crytosol, showed an entirely different behavior. After treatment it retained most of its activity, and upon storage there was a gradual decrease in activity.

Numerous reactions on proteins can take place when processed by physical or chemical means. These reactions induce undesirable changes in living cells and may decrease the nutritional value and organoleptic quality of a protein-containing food. They occur often through the formation of covalent cross-links between polypeptide chains (Cheftel, 1977). Red cell membranes have been proposed as a model for protein damage in tissues where proteins and lipids are organized in a well-defined manner.

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

Several different biochemical mechanisms have been implicated in the polymerization of red cell membrane proteins. Lorand et al. (1976) described a calcium-activated transglutaminase that catalyzed the cross-linking through the formation of γ-glutamyl-ϵ-lysine bridges. A second mechanism is the oxidation of sulfhydryls to form intermolecular disulfide bridges. Another explanation for the membrane protein cross-linking observed is Schiff base formation by the reaction of amino groups with malonaldehyde, an end product of polyunsaturated fatty acid peroxidation present in the membrane as various phospholipids (Tappel, 1973), although other reactions are possible, e.g., direct radical attack on the proteins (Hochstein and Jain, 1981).

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